

Serial No.: 09/782,386

Filed: February 12, 2001

computer readable sequence listing, and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph starting on page 9, line 25, has been amended as follows:

- An oligonucleotide (oligo #1, SEQ ID NO: 1) comprising nucleotides 374-403 of the E6 gene of human papilloma virus type 16 was synthesized by the phosphoramidite method of DNA synthesis and labeled with ³²P at the 5' end. A second ³²P-labeled oligonucleotide (oligo #2, SEQ ID NO: 2) containing the same sequence as oligo #1 (SEQ ID NO: 1) except for a single G->A base change at position 388 was also prepared.

Oligo #1, SEQ ID NO: 1: 5'-CAA TAC AAC AAA CCG TTG TGT GAT TTG TTA-3'

Oligo #2, SEQ ID NO: 2: 5'-CAA TAC AAC AAA CCA TTG TGT GAT TTG TTA-3' -

The paragraph starting on page 9, line 32, has been amended as follows:

-A 20-mer DNA probe (oligo #3, SEQ ID NO: 3) containing the photoactive cross-linking group, 3-O-(7-coumarinyl) glycerol (denoted by ~~XN~~ in the sequence) was prepared. This DNA sequence of this probe is fully complementary to oligo #1 (SEQ ID NO: 1) but would hybridize with oligo #2 (SEQ ID NO: 2) to form a duplex containing an A/C mismatch.

Oligo #3, SEQ ID NO: 3: 3'-TTG TTT GGC AAC ACA CTA ~~XNA~~-5'

Oligo #1/#3 duplex:

SEQ ID NO: 1 5'-CAA TAC AAC AAA CCG TTG TGT GAT TTG TTA-3'

SEQ ID NO: 3 3'-TTG TTT GGC AAC ACA CTA ~~XNA~~-5'

Oligo #2/#3 duplex:

SEQ ID NO: 2 5'-CAA TAC AAC AAA CCA TTG TGT GAT TTG TTA-3'

SEQ ID NO: 3 3'-TTG TTT GGC AAC ACA CTA ~~XNA~~-5' -

The paragraph starting on page 10, line 10, has been amended as follows:

--Oligo #3, SEQ ID NO: 3 (20 pmole) was incubated in the presence of 2 pmole of either ³²P-5' end-labeled oligo #1 (SEQ ID NO: 1) or oligo #2 (SEQ

ID NO: 2) in 0.15 mL samples at the temperatures and NaCl concentrations summarized below:-

The paragraph starting on page 11, line 1, has been amended as follows:

--By carrying out the experiment under a range of hybridization temperatures (45-55°C) and NaCl concentration (150-300 mM), it was possible to define conditions that led to appreciable cross-link formation between the complementary oligonucleotides #1 (SEQ ID NO: 1) and #3 (SEQ ID NO: 3) but not the mismatched oligonucleotides #2 (SEQ ID NO: 2) and #3 (SEQ ID NO: 3). To determine the best conditions for mismatch discrimination the radioactive bands were excised from the gel, quantified by scintillation counting and the percent yield of cross-linked product measured (relative to unreacted ³²P-labeled oligonucleotide). The results are shown below:--

The paragraph starting on page 11, line 32, has been amended as follows:

--Analysis of the autoradiogram for the samples (3 and 4) run under the least stringent hybridization conditions (45°C, 300 mM NaCl) clearly showed that the product obtained from cross-linking between the mismatched oligonucleotides #2 (SEQ ID NO: 2) and #3 (SEQ ID NO: 3) migrated slower through the gel than the product obtained from cross-linking the complementary oligonucleotides #1 (SEQ ID NO: 1) and #3 (SEQ ID NO: 3) (the DSCP effect).--

The paragraph starting on page 12, line 7, has been amended as follows:

--2. By using non-stringent conditions the cross-link yield and hence the signal in the assay is higher than when the hybridization stringency method is employed; under the conditions used for DSCP analysis (45°C, 300 mM NaCl) the cross-link yield for the reaction between the complementary oligonucleotides #1 (SEQ ID NO: 1) and #3 (SEQ ID NO: 3) was 49%, however under the conditions that led to the best mismatch discrimination with the hybridization stringency method (55°C, 150 mM NaCl), the cross-linking efficiency was 39%. Thus the DSCP method resulted in 26% greater signal.--

The paragraph starting on page 12, line 18, has been amended as follows:

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--Two 56 base oligonucleotides comprising a portion of the sequence of either the normal human β -globin gene (β^A -target) or the sickle cell β -globin gene (β^S -target) were synthesized by the phosphoramidite method of DNA synthesis and labeled with ^{32}P at their 5' ends. The β^S -globin target sequence differs from the β^A -target by a single A->T mutation that gives rise to a mutant β -globin protein that contains valine instead of glutamic acid.

β^A -target: 5'-TGA CTC CTG AGG AGA AGT CTG CCG TTA CTG CCC TGT-
GGG GCA AGG TGA ACG TGG AT-3' (SEQ ID NO: 4)

β^S -target: 5'-TGA CTC CTG TGG AGA AGT CTG CCG TTA CTG CCC TGT-
GGG GCA AGG TGA ACG TGG AT-3' (SEQ ID NO: 5)--

The paragraph starting on page 12, line 28, has been amended as follows:

--Two probes complementary to either the β^A -target sequence (β^A -probe) or the β^S -target (β^S -probe) were also synthesized. These probes were modified with the photoactive cross-linking group, 3-O-(7-coumarinyl) glycerol (denoted by *N in the sequence):

β^A -probe: 3'-TGA GGA CTC CTC TTC A*NNA-5' (SEQ ID NO: 6)

β^S -probe: 3'-TGA GGA CAC CTC TTC A*NNA-5' (SEQ ID NO: 7)--

On page 15, immediately preceding the claims, the enclosed text entitled "SEQUENCE LISTING" was inserted into the text.